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The work reported here was done under contract no. IAC-00027-D, Federal Aviation Administration, Civil Aeronautical Institute, Oklahoma City, Oklahoma, and Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio 45433. **THE ACUTE TOXICITY OF BRIEF EXPOSURES TO HF, HCl, NO, AND HCN SINGLY AND IN COMBINATION WITH CO** by E. Arnold Higgins, Ph.D.; Vincent Florida, Ph.D.; A. A. Thomas, M. D.; and Harvey V. Davis, Ph.D. November 1971, pp. 8 Report No. FAA-AM-71-41.

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AUTHORS

E. Arnold Higgins, Ph.D., Civil Aeromedical Institute, Federal Aviation Administration, Oklahoma City, Oklahoma.

Vincent Fiorica, Ph.D., Civil Aeromedical Institute, Federal Aviation Administration, Oklahoma City, Oklahoma.

A. A. Thomas, M.D., Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio 45433.

Harvey V. Davis, Ph.D.,* Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio 45433.

*Harvey V. Davis, Ph.D., now employed with Standard Oil Company of Illinois, Department of Industrial Hygiene and Toxicology, 910 S. Michigan Ave., Chicago, Illinois 60686.

THE ACUTE TOXICITY OF BRIEF EXPOSURES TO HF, HCl, NO₂ AND HCN SINGLY AND IN COMBINATION WITH CO

I. Introduction.

Recent studies made within the aviation community illustrate the importance of fire, smoke, and fumes in the survivability and escape from aircraft accidents. For example, the Aerospace Industries Association (AIA)¹ examined the importance of fire in 530 fatalities which occurred in 16 impact-survivable accidents. They concluded that fatalities in 12 of these accidents were the result of the ensuing fire. Another report² showed that during the period 1955-1964, 12 survivable U. S. Air Carrier accidents occurred in which 68 crew members and 596 passengers either died by fire or received severe injuries attributable to fire. The same study also showed that 25 crew members and 269 passengers died by fire in other accidents which had some fatalities at impact; the deaths by fire occurred post-impact. Snow, *et al.*,³ made an exhaustive analysis of the human factors data from three aircraft accidents involving emergency evacuation. All three accidents were impact-survivable but 105 of the 261 passengers died by fire or smoke during attempts to escape the aircraft after impact. Hasbrook, *et al.*,⁴ examined, in some detail, the evacuation pattern of a survivable accident in which 16 passengers died from smoke inhalation and fire.

Comprehensive aircraft safety necessitates the rapid evacuation of passengers immediately following impact-survivable crashes. Many such crashes result in aircraft fires. Since inhalation of the combustion products of aircraft components may present a general toxicity hazard, it would be wise to select totally nonflammable materials or, lacking such materials, to select those having the least toxic combustion products.

Marcey and Johnson of NAFEC have conducted studies⁵ of the burning characteristics of a total of 140 different materials divided into two groups; one group comprised materials now in use in air transport, the other, materials which

have been proposed for future use and which display superior resistance to fire. Also, the Fire Research Section of the National Bureau of Standards conducted research for NAFEC on the smoke and gases produced by burning materials used in the interior of aircraft.⁶ Neither test, however, examined the biological effects of the toxic combustion products found.

Of the interior materials presently in use, many formulations contain halogen, cyanide, and nitrogenous moieties which can react during combustion or pyrolysis to form the corresponding halogen acid gases (HCl and HF), hydrogen cyanide gas (HCN), and nitrogen dioxide (NO₂). Information has been needed concerning the toxicity of these materials when exposure is for a short period of time as might be experienced in the evacuation of a burning aircraft. The present study was conducted to provide this specific information for the gases HCl, HF, HCN and NO₂. In addition, other experiments were performed to explore the toxic effects of a simultaneous exposure to each of the above gases in combination with carbon monoxide (CO).

This study is not an exhaustive evaluation of the toxicity of pyrolysis products. It was designed to establish some criteria for evaluating material in use, or proposed for use, in terms of some of the more commonly produced, easily identifiable toxic gases.

II. Experimental Approach.

Ten rats (Wistar) and 15 mice (ICR) per group were exposed to a series of atmospheric concentrations of each test material to determine LC₅₀ (Lethal Concentration for 50% of the test animals) values. Exposed rats weighed from 250 to 275 grams, while mice ranged from 30 to 35 grams. Quality control examinations were conducted on each shipment of rats and mice received to assure that healthy animals were used in the toxicity studies.

The animals were exposed in a dynamic flow system using a standard Rochester chamber⁷ modified to present the animals with a precisely-timed 5-minute inhalation challenge. The modification consisted of a cage constructed with gasketed solid ends mounted on a slide track which was installed in one of the plastic panels on the Rochester chamber. When the desired contaminant concentration was achieved in the chamber, the cage containing animals was rapidly pushed into the chamber and the sealing clamps were secured. After five minutes of exposure the procedure was reversed and another experiment was started by readjusting the contaminant concentration.

The animals were observed closely for seven days following exposure in order to include among the observations any delayed deaths which were due to pulmonary edema.

A series of animal exposures was conducted to determine the concentration of CO required to produce 25% COHb (a level which produces

minor CNS effects in man but which is not lethal *per se*). Initial experimental CO concentrations were based on calculations made from the human CO uptake data of Forbes, *et al.*⁸ Blood carboxyhemoglobin measurements were made on pooled blood samples from simultaneously-exposed groups of three or four rats, using the method of Goldbaum, *et al.*⁹ The COHb data for rats are shown in Figure 1. Also shown in this figure is the average COHb value for 24 mice exposed to a single carbon monoxide concentration. Since all animal species have from 0.5 to 1.0% endogenous COHb from hemoglobin catabolism, this is essentially a 2-point plot showing that the 25% COHb level is achieved in mice during five minutes of exposure to 1,500 ppm CO. The 5-minute CO concentration required to achieve 25% COHb in rats is 2,100 ppm.

All contaminant exposure concentrations were controlled by continuous monitoring. Hydrogen fluoride, HCl, and HCN concentrations were monitored using specific ion electrodes. Air samples were absorbed into a constant flow of aqueous reagent solutions made in accordance with the directions of the electrode manufacturer for pick-up of the contaminant and optimum measurement of the ion. Air and solution flow rates were measured precisely for conversion of ion concentration to airborne contaminant concentration. Calibration curves were prepared by sampling known concentrations of the specific ions made from primary standards. The standards used were NaF, NaCN, and NaCl.

Nitrogen dioxide was analyzed by continuous spectro-photometric measurement of atmospheric samples absorbed in Saltzman reagent using an AutoAnalyzer.¹⁰ Calibration curves were prepared by analytical measurement of standard gas bag concentrations backed up by standardization with permeation tubes.¹¹

The LC₅₀ values for each series of exposure to the compounds tested, either singly or in combination with CO, were calculated by the method of Litchfield and Wilcoxon¹² using computer program techniques. This method results in a slope calculated by the method of least squares which results in the lowest Chi square value possible.

III. Results and Discussion.

Hydrogen fluoride produced pulmonary edema of varying degrees of severity in most of the

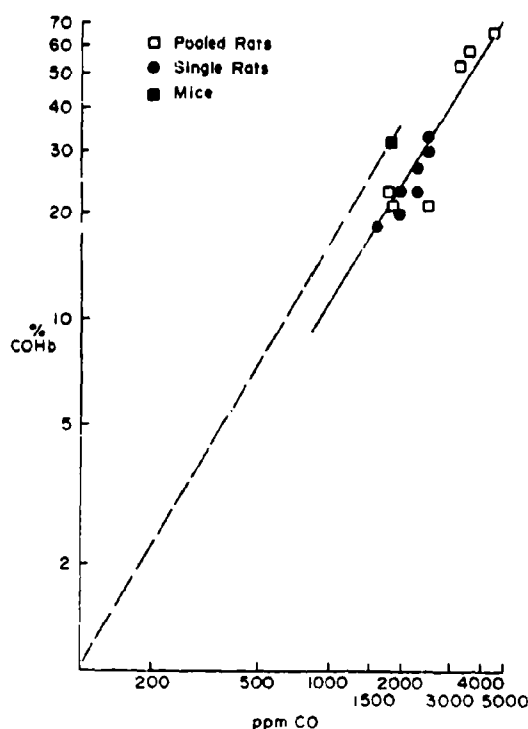


FIGURE 1. Carboxyhemoglobin Formation in Rodents Exposed to CO for 5 Minutes.

exposed animals. In animals that died during or shortly after exposure to concentrations above the LC_{50} value, pulmonary hemorrhage was a common finding. Delayed deaths were routinely seen with this compound in exposures below the LC_{50} level, with peak mortality occurring about 24 hours postexposure, although occasional deaths occurred three to four days later. The mortality rates to inhaled HF for rats are presented in Table 1; those rates for mice are in Table 2. The LC_{50} slopes for rats and mice are presented in Figures 2 and 3, respectively. The slopes for the HF and HF + CO exposures are not statistically different. There is no apparent effect attributable to concurrent exposure to carbon monoxide. Indeed, a single slope could be plotted by combining the two sets of exposure data, which would result in a more precise LC_{50} value with narrower 95% confidence limits.

Exposure of rats to HCl resulted in LC_{50} values of 40,989 ppm for the pure compound, and 39,010 ppm for the combined exposure of HCl and CO, as shown in Table 3. This difference was not statistically significant; neither was the greater difference found in mice as shown in Table 4. The LC_{50} slopes are plotted in Figure 4 for rats and in Figure 5 for mice. Hydrogen

TABLE 1.—7-Day Mortality Response of Rats Exposed 5 Minutes to HF Gas Singly and in Combination with CO (25% Carboxyhemoglobin)

HF Concentration (ppm)	% Deaths	
	HF	HF + CO
11,550		0
12,440	10	0
12,890		10
15,990		40
17,615	30	
17,750		100
18,580	80	90
20,730	70	100
21,125		100
22,355		100
22,740		100
23,540		100
25,690	100	
LC_{50}	18,200 ppm	18,208 ppm
95% Confidence Limits	15,965-20,748 ppm	13,698-24,202 ppm

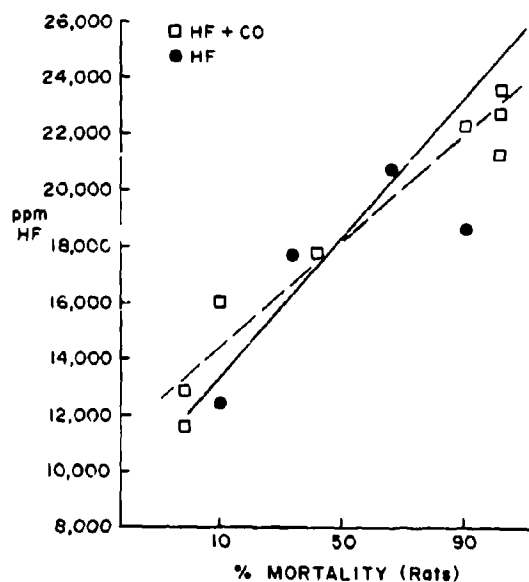


FIGURE 2. Five Minute LC_{50} for Rats Exposed to HF Singly and in Combination with CO.

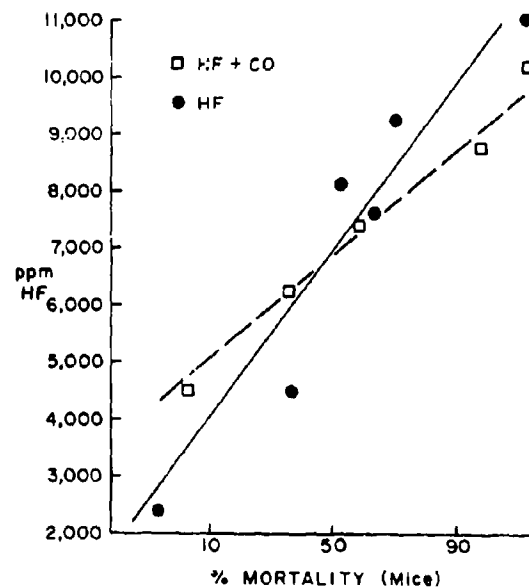


FIGURE 3. Five Minute LC_{50} for Mice Exposed to HF Singly and in Combination with CO.

chloride is definitely less toxic to rats than is HF, but, in mice, the effective range of lethal response to HCl overlaps that of HF. It should be noted that the range of HCl concentrations which had to be used was much wider than that with HF.

TABLE 2.—7-Day Mortality Response of Mice Exposed 5 Minutes to HF Gas Singly and in Combination with CO (25% Carboxyhemoglobin)

HF Concentration (ppm)	% Deaths	
	HF	HF + CO
2,430	0	7
4,480		
4,500	33	
6,220		33
7,410		60
7,615	67	
8,140	53	
8,760		93
10,190		100
11,010	100	
LC ₅₀	6,247 ppm	6,670 ppm
95% Confidence Limits	4,789-8,140 ppm	5,690-7,807 ppm

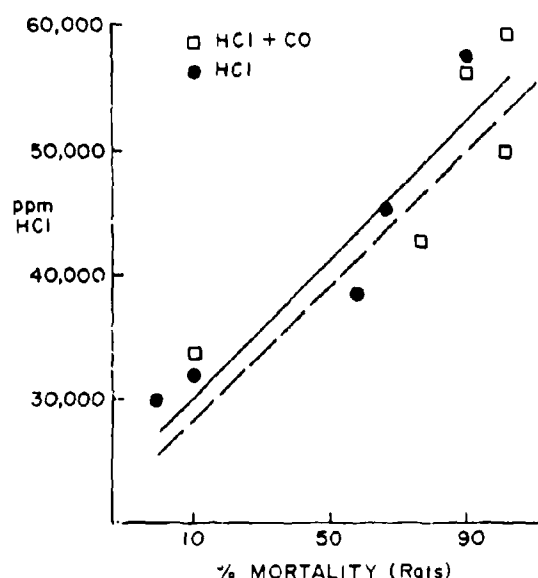


FIGURE 4. Five Minute LC₅₀ for Rats Exposed to HCl Singly and in Combination with CO.

TABLE 3.—7-Day Mortality Response of Rats Exposed 5 Minutes to HCl Vapors Singly and in Combination with CO (25% Carboxyhemoglobin)

HCl Concentration (ppm)	% Deaths	
	HCl	HCl + CO
30,000	0	
32,000	10	
33,980		10
39,850	60	
42,460		80
45,200	70	
49,580		100
56,046		90
57,290	90	
59,280		100
LC ₅₀	40,989 ppm	39,010 ppm
95% Confidence Limits	34,803-48,272 ppm	35,049-43,419 ppm

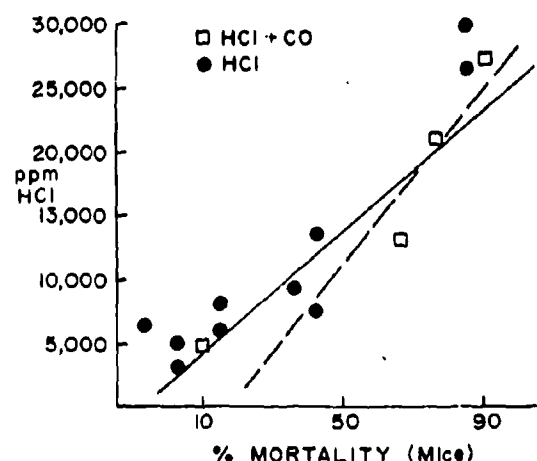


FIGURE 5. Five Minute LC₅₀ for Mice Exposed to HCl Singly and in Combination with CO.

Consequently, the 5-minute toxicity of HCl is much less predictable than that of HF. The lowest concentration of HCl causing death in mice was 3,200 ppm; however 100% deaths were not achieved at the highest concentration tested (30,000 ppm), which was an order of magnitude greater.

Most NO₂-induced animal deaths resulted from pulmonary edema and were seen within 12 hours postexposure; a few animals exhibited pulmo-

TABLE 4.—7-Day Mortality Response of Mice Exposed 5 Minutes to HCl Gas Singly and in Combination with CO (25% Carboxyhemoglobin)

HCl Concentration (ppm)	% Deaths	
	HCl	HCl + CO
3,200	7	10
4,920		
5,060	7	
6,145	13	
6,410	0	70
7,525	40	
8,065	13	
9,278	33	
12,805		80
13,655	40	
21,010		
26,485	87	
27,386		90
30,000	87	
LC ₅₀	13,745 ppm	10,663 ppm
95% Confidence Limits	10,333-18,283 ppm	6,021-16,428 ppm

TABLE 5.—7-Day Mortality Response of Rats Exposed 5 Minutes to NO₂ Singly and in Combination with CO (25% Carboxyhemoglobin)

NO ₂ Concentration (ppm)	% Deaths	
	NO ₂	NO ₂ + CO
260	10	0
270		
550	40	
580		
590	30	20
750		
840	30	
850	30	
1,000		40
1,200	50	
1,250	90	
1,380	100	
1,660		80
LC ₅₀	831 ppm	1,140 ppm
95% Confidence Limits	558-1,240 ppm	720-1,707 ppm

nary hemorrhage. Again, as shown in Tables 5 and 6, there was no significant difference between exposure to NO₂ alone and exposure to NO₂ in combination with CO. Simultaneous exposure of rats to CO and NO₂ resulted in a slightly higher LC₅₀, an indication of a slightly less toxic response. The results of these LC₅₀ studies of NO₂ on rats are almost identical to those reported by Gray, *et al.*¹³; the latter found a 5-minute LC₅₀ value of 832 ppm which compares well with our value of 831 ppm.

In HCN exposures, either singly or in combination with CO, all deaths occurred during the exposure period or within 20 minutes post-exposure. There were no delayed deaths. The results of the experiments are given in Tables 7 and 8 for rats and mice, respectively. Carbon monoxide at the 25% carboxyhemoglobin level had no effect on the acute toxicity of HCN. Because the primary effect of HCN intoxication is the blocking of intracellular oxygen transport through the cytochrome system, the slightly decreased extra-cellular oxygen transport was not

TABLE 6.—7-Day Mortality Response of Mice Exposed 5 Minutes to NO₂ Singly and in Combination with CO (25% Carboxyhemoglobin)

NO ₂ Concentration (ppm)	% Deaths	
	NO ₂	NO ₂ + CO
260	7	0
550	27	
580		
590	0	
840	7	7
850	0	
950		
1,200	40	
1,250	13	58
1,380	47	
1,500		
1,990	20	
2,280		74
2,560	67	
2,950	74	
2,980	100	
3,280		98
LC ₅₀	1,886 ppm	1,644 ppm
95% Confidence Limits	1,345-2,828 ppm	1,203-2,247 ppm

TABLE 7.—7-Day Mortality Response of Rats Exposed 5 Minutes to HCN Singly and in Combination with CO (25% Carboxyhemoglobin)

HCN Concentration (ppm)	% Deaths	
	HCN	HCN + CO
280		0
283	0	
334		10
357	10	
368	20	
407	20	
504		70
557		70
583	80	
690	100	
LC ₅₀	503 ppm	467 ppm
95% Confidence Limits	403-626 ppm	395-553 ppm

TABLE 8.—7-Day Mortality Response of Mice Exposed 5 Minutes to HCN Singly and in Combination with CO (25% Carboxyhemoglobin)

HCN Concentration (ppm)	% Deaths	
	HCN	HCN + CO
188		0
200	0	
283	27	
288		61
300		40
319		87
357	80	
360		87
368	67	
414	80	
427	100	
LC ₅₀	323 ppm	289 ppm
95 % Confidence Limits	276-377 ppm	245-340 ppm

an important factor. In aircraft fires, it is possible that the build up of CO will be faster than the build up of other toxic gases. In order to determine the effect of prior exposure to CO on mortality rates of HCN, two separate groups of rats were exposed to HCN immediately fol-

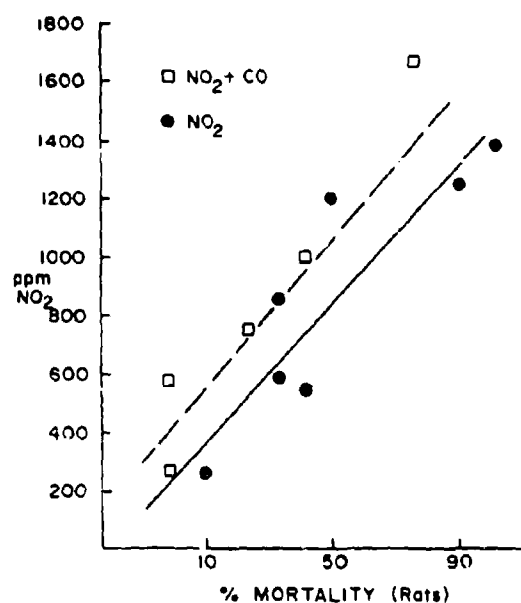


FIGURE 6. Five Minute LC₅₀ for Rats Exposed to NO₂ Singly and in Combination with CO.

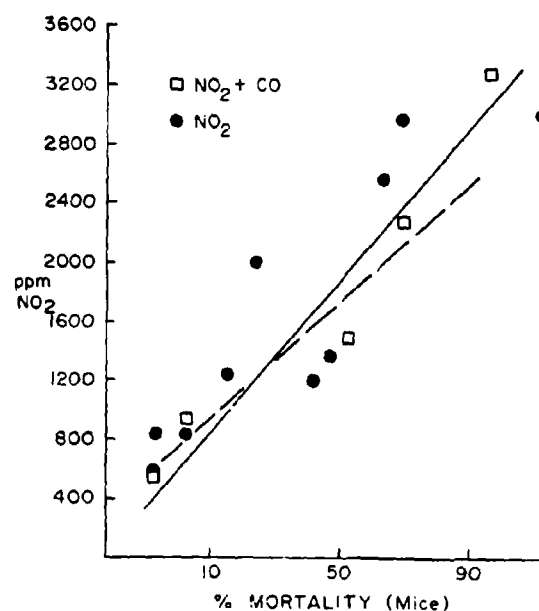


FIGURE 7. Five Minute LC₅₀ for Mice Exposed to NO₂ Singly and in Combination with CO.

lowing exposure to CO. The exposure to CO resulted in 25% COHb for one group of rats and 50% COHb for the second group. The results of these exposures were no different from those in which the CO and HCN were used simultaneously. The data are presented graphically in Figure 8 for rats, and in Figure 9 for mice

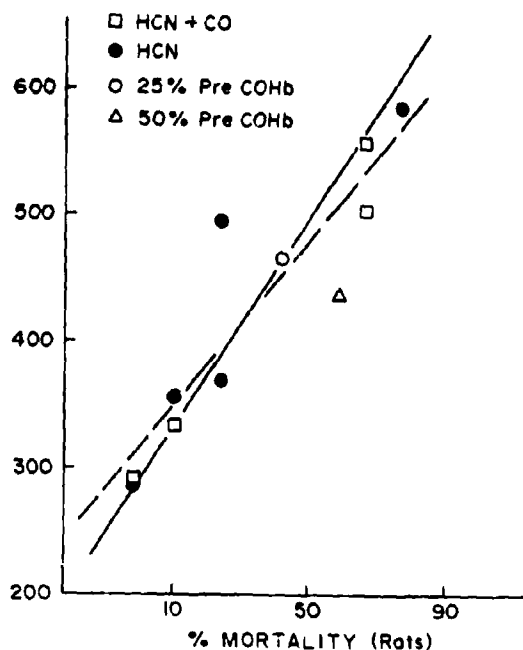


FIGURE 8. Five Minute LC_{50} for Rats Exposed to HCN Singly and in Combination with CO.

The CO exposure superimposed on the individual contaminant exposures might have resulted in more rapid responses, i.e., a decrease time to death. Such a response would be important in consideration of potential hazards for aircraft passengers. We compared the mortality time data of single and combined exposures and found no significant differences or trends.

These acute studies show that the toxicity ranking of the four materials tested is HCN, NO_2 , HF, and HCl, in decreasing order. How-

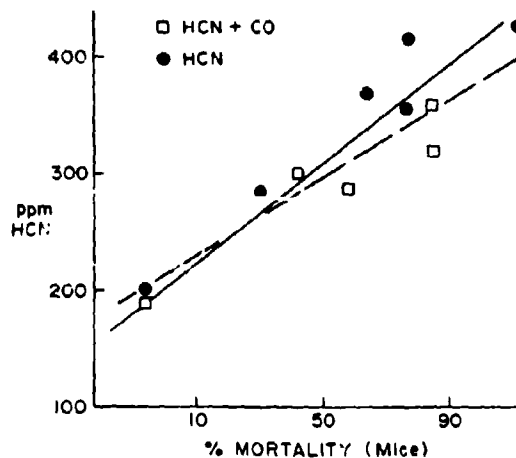


FIGURE 9. Five Minute LC_{50} for Mice Exposed to HCN Singly and in Combination with CO.

ever, the hazard rating of various plastic formulations would require experimental determination of the amounts of these materials produced by pyrolysis of comparable quantities (i.e., although the combustion of 100 pounds of a cyanide-containing plastic might produce concentration of 200 ppm HCN in an aircraft cabin, it is possible that the combustion of some chloride-producing plastic might yield cabin concentrations as high as 50,000 ppm; in this event the chloride-containing plastic would present the greater hazard).

We have shown in these experiments that carbon monoxide concentrations which are not hazardous to life do not enhance the toxic response to the four substances tested. Some LC_{50} values for CO combined with another toxic compound are slightly lower than those of the compounds alone. However, the series of exposures of rats to NO_2 with 25% carboxyhemoglobin established prior to exposure indicates that there is neither a trend toward enhancement of toxicity, nor a protective value, but that the results vary randomly to the extent which one would expect from repeated series of 5-minute LC_{50} values for the various compounds.

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